Final Report

Original 2 of 2

Determination of 24h- and 48h-$EC50_i$ of [redacted]

against *Daphnia magna* STRAUS
according to OECD 202 resp. EU C.2

Study No. [redacted]

Sponsor: [redacted]

Test Facility: [redacted]

Monitor: [redacted]

Study Director: [redacted]

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1 GLP-Compliance Statement

It is hereby declared that all tests were made in accordance with the „Revised OECD Principles of Good Laboratory Practice" (Paris, 1997) as stated in the following guidelines:

- OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997; Environment Directorate, Organisation for Economic Cooperation and Development, Paris 1998
- Directive 2004/10/EC of the European Parliament and of the Council of 11 February 2004 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances (codified version)
- Chemikaliengesetz (Chemicals Act) of the Federal Republic of Germany (ChemG) § 19(a) to §19(d) and annexes 1 and 2, version 02 July 2008, Federal Law Gazette, Germany (BGBL) N. 28/2008, pp. 1146-1184, amended in Federal Law Gazette, Germany (BGBL) from 02 November 2011, N. 56/2011, pp. 2162-2169

Responsibility for the accuracy of the information concerning the test item as well as for its authenticity rests with the sponsor.
I herewith accept responsibility for the data presented within this report.
There were no circumstances that may have affected the quality or integrity of the study.

The following parameters were not determined under GLP conditions:
Range-finding test (performed before preparation of the study plan):

[redacted]

[redacted]

Information on Study Organisation:

Deputy Study Director

Study Plan dated

Experimental Starting Date

Experimental Completion Date

Draft Report dated

[redacted]

2 QUALITY ASSURANCE UNIT STATEMENT

This study has been inspected by the quality assurance unit according to the principles of Good Laboratory Practice. Study Plan and Final Report were checked at the dates given below, the Study Director and the management were informed with the corresponding report.

Also, the performance of the study was inspected, and findings were reported to Study Director and management. The inspection of short-term studies (duration less than four weeks) is carried out as audit of process concerning major technical phases of at least one similar test. Frequency is once or more a quarter.

The study was conducted and the reports were written in accordance with the Study Plan and the Standard Operating Procedures of the test facility.

Deviations from the Study Plan were acknowledged and assessed by the Study Director and included in the Final Report.

The reported results reflect the raw data of the study.

Verified Procedure	Inspected on	Findings reported on	Audit report no.
Study plan	[redacted]		
Performance of study			
Draft report			
Final report			

[redacted]

Date [redacted]

Table of Contents

3 SUMMARY

Title of Study: Determination of 24h- and 48h-$EC50_i$ of [redacted] against *Daphnia magna* STRAUS according to OECD 202 resp. EU C.2

Findings and Results:

As the test item is [redacted] in water, a saturated solution was prepared. This was done by shaking the nominal load with the appropriate amount of dilution water for 24 hours, followed by membrane filtration. The concentrations to be tested were prepared by dilution of this solution with dilution water.

The study was performed using five concentrations ranging from 10 to 100 mg/L nominal concentration. Twenty daphnia were exposed to the test item for 48 hours in a static test system. After 24 and 48 hours, the immobilised daphnia were counted.

Only the highest concentrated treatment showed significant immobilisation (45 %). None of the animals were immobilised in the control.

The 24h-$EC50_i$ of potassium dichromate was tested in a current reference test. The value was determined as 1.7 mg/L, lying within the demanded range of 0.6 – 1.7 mg/L.

At the beginning and at the end of the test, the content of [redacted] component of the test item) in the test solution was analytically determined using AAS. Because of the [redacted] in test medium, the correlation between nominal and measured concentration was poor. Therefore, the determination of the results was based on the geometric mean of the measured concentrations

The following results were determined for the test item [redacted] (species: *Daphnia magna*).

24h-NOEC ≥ 42 mg/L
48h-NOEC = 24 mg/L
24h-$EC50_i$ > 42 mg/L
48h-$EC50_i$ > 42 mg/L

42 mg/L [redacted]

4 PURPOSE AND PRINCIPLE OF THE STUDY

This study was performed in order to evaluate the toxic potential of [redacted] towards freshwater shrimp, using the species *Daphnia magna*. *Daphnia magna* STRAUS, which belongs to the family of crustacea, was chosen in the guideline as a typical part of zooplancton.

Young daphnia, aged less than 24 hours at the start of the test, were exposed to the test item at a range of concentrations for a period of 48 hours. Immobilisation was recorded at 24 hours and 48 hours and compared with control values. The results were analysed in order to calculate the EC50 at 48h and 24h.

A positive control was tested for EC50 as a means of assuring that the test conditions are reliable.

Sponsor's intent: Notification in accordance with: REACH.

5 LITERATURE

The study was conducted in accordance with the following guidelines:

- OECD Guideline for Testing of Chemicals No. 202, adopted 13. Apr. 2004: "Daphnia sp., Acute Immobilisation Test"
- Commission Regulation (EC) No. 440/2008, Method C.2. "Daphnia sp. Acute Immobilisation Test", adopted 31. May 2008
- EN ISO 6341 "Bestimmung der Hemmung der Beweglichkeit von Daphnia magna Straus (Cladocera, Crustacea) – Akuter Toxizitätstest" as of June 1996

Corresponding SOP of [redacted]

- [redacted]

6 MATERIALS AND METHODS

6.1 Test Item

Designation in Test Facility: [redacted]
Date of Receipt:
Condition at Receipt

6.1.1 Specification

The following information concerning identity and composition of the test item was provided by the sponsor.

Name [redacted]
Batch no.
Appearance
Composition

CAS No.
EINECS-No.
Molecular formula
Molecular weight
Purity
Homogeneity
Vapour pressure
Stability
Solubility
Production date
Expiry date
Storage
Hazard information
R-phrases
S-phrases

6.1.2 Storage

The test item was stored in a closed vessel dark and dry at room temperature.

6.1.3 Preparation

As solubility lies below [redacted] the saturated solution was prepared for the test. This was done by weighing the nominal load, adding the corresponding amount of dilution water and shaking vigorously for 24 hours. The resulting solution was filtrated through 0.45 µm filters.

6.2 Positive Control

Potassium dichromate $K_2Cr_2O_7$ (CAS No. 7778-50-9) was used as positive control.

6.3 Test System

6.3.1 Specification

Species	*Daphnia magna*
Variety	STRAUS
Strain	Berlin
Sex	female
Age	between 0 and 24 hours
Origin	Umweltbundesamt Berlin
Arrival of Strain	27. Sep. 2007

Selection of the test system was made following the proposal of the guidelines.

6.3.2 Animal Husbandry

Daphnia magna is bred in the [redacted] throughout the year. The animals are kept for the use in toxicity tests. They multiply by parthenogenesis, thus being genetically identical. The husbandry is performed similar to the method described in EN ISO 6341, following [redacted]

Vessels	glass beakers, nominal volume 2 l
Medium	M4-Medium (recipe of ELENDT), composition see annex
Food	unicellular green algae (*Desmodesmus subspicatus*)
Medium renewal	twice a week
Photo period	16/8 hours, using neon tubes
Temperature	20 ± 2 °C

6.4 Dilution Water

Deionised water with an enrichment of certain minerals (as demanded in the guidelines) is used in the test.

Table 6.4-a Dilution water specification

Parameter	Concentration in mg/L
$CaCl_2*2H_2O$	293.80
$MgSO_4*7H_2O$	123.30
$NaHCO_3$	64.80
KCl	5.80
Resulting hardness in mmol/L:	2.502
Resulting hardness in mg $CaCO_3$/L:	250

After preparation, the dilution water is aerated, and the pH is adjusted to 7.8 ± 0.2.

6.5 Test Vessels

Beakers, glass, nominal volume 50 mL.

6.6 Instruments and Devices

The following instruments and devices were used in the performance of the study:

- Data logger for temperature
- Analytical scales Mettler XS 205 DU No. 2
- Adjustable pipettes with one-way tips, [redacted]
- Glass measuring cylinders
- Glass measuring flasks
- pH-meter 340i wtw
- Oxygen meter Oxical 340i wtw
- Orbital Shaker GFL 3019 [redacted]
- AAS contrAA 300 S

Usage and, if applicable, calibration of all instruments following the corresponding SOP in the current edition.

Standard laboratory equipment (glassware) was also used.

6.7 Analytical Method

The content of the test item [redacted] in the test solutions was determined by measurement of [redacted] as a component of the test item [redacted] using AAS. AAS measurements were performed with the flame technique (Air/N_2O) at [redacted] nm for [redacted]

6.7.1 Sample Preparation

To each sample, 100 µL KCl (10% in water) and 100 µL HCl conc. were added. For the concentration 10 mg/L and 18 mg/L and the control the mixture was filled up to 10 ml with test solution respectively control solution. Then, [redacted] was measured in the C_2H_2/air flame at [redacted] nm. For the concentration 100 mg/L test item, a 1:10, for 56 mg/L a 1:5 and for 32 mg/L a 1:2 dilution was performed. 1000 µL, 2000 µL and 5000 µL test item solution was filled up to 10 mL with demineralised water after addition of 0.1 ml KCl (10% in water) and 0.1 mL HCl conc.

6.7.2 Parameters of Instrument

Specification: AAS contrAA 300, Analytik Jena AG
Absorption line [redacted]

6.7.3 Method Characterisation

Calibrated range: 0.01 – 2 mg/[redacted]
Variation coefficient of method 1.5 % (calibration 29.11.2011)
3.04 % (calibration 01.12.2012)
Limit of detection [redacted] 0.01 mg/L [redacted]
Limit of quantification [redacted] 0.01 mg/L [redacted]
The lowest concentration of the calibration 0.01 mg/L [redacted] was stated as limit of determination and quantification.

6.7.4 Accuracy

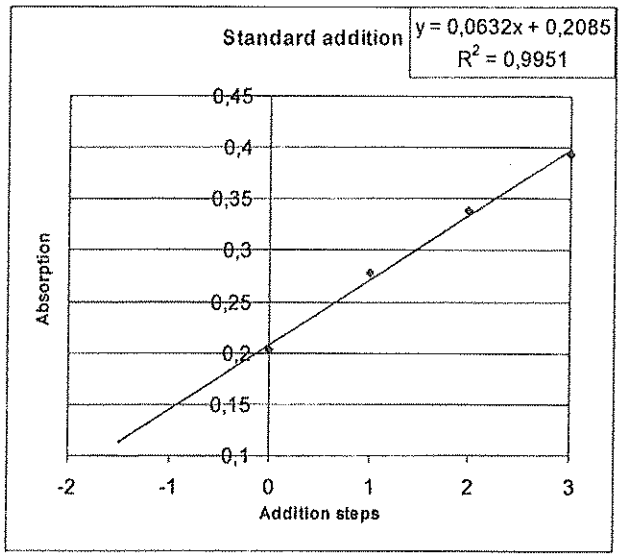
The accuracy in daphnia medium was determined via standard addition. To 11.6 mg test item, 100 mL Daphnia test medium were added and the mixture was shaken for 24 hours at room temperature. After shaking, the turbid solution was membrane filtrated (0.45 µm), 5 mL of the filtrate was given in a 50 mL measured flask, 500 µL KCl (10% in water) and 500 µL HCl conc. were added and the flask was filled up to 50 mL with demineralised water (dilution 1:10).

Four 10 mL measuring flasks were prepared. Each flask was filled up to 10 mL with a diluted test item solution after addition of 0, 250 µL; 500 µL; 750 µL (respectively) of [redacted] stock solution (10 mg/L). The samples were measured in triplicate.

Table 6.7-a Results Standard Addition Daphnia test medium

Addition	Amount of [redacted] stock sol.	Measured value (mean)	Measured conc. (mg[redacted]L)	Parameter of regression	Value
0	-	0.20322	0.477	Slope	0.063217
1	250 µl	0.27763	0.689	Intercept	0.208457
2	500 µl	0.33869	0.883	r	0.997563108
3	750 µl	0.39359	1.075	r^2	0.995132154

Graph of Standard Addition in Daphnia test medium:



6.7.5 Assessment

Conditions for the accuracy

Correlation coefficient r ≥ 0.996

The condition is fulfilled for Daphnia test medium with correlation coefficient r = 0.9976.

6.7.6 Stability of Solutions

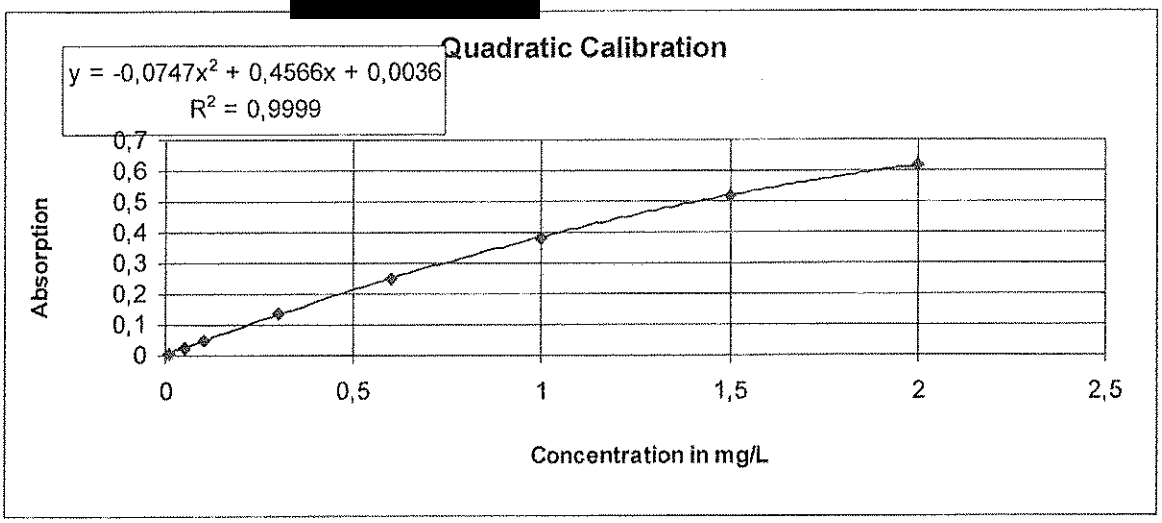
The recovery rate for the test item at a concentration of 100.1 mg/L (nominal) after 48 hours was determined as 105 %. Thus the test item can be stated as stable under test conditions. The details are given in the following table.

Table 6.7-b Recovery Rates

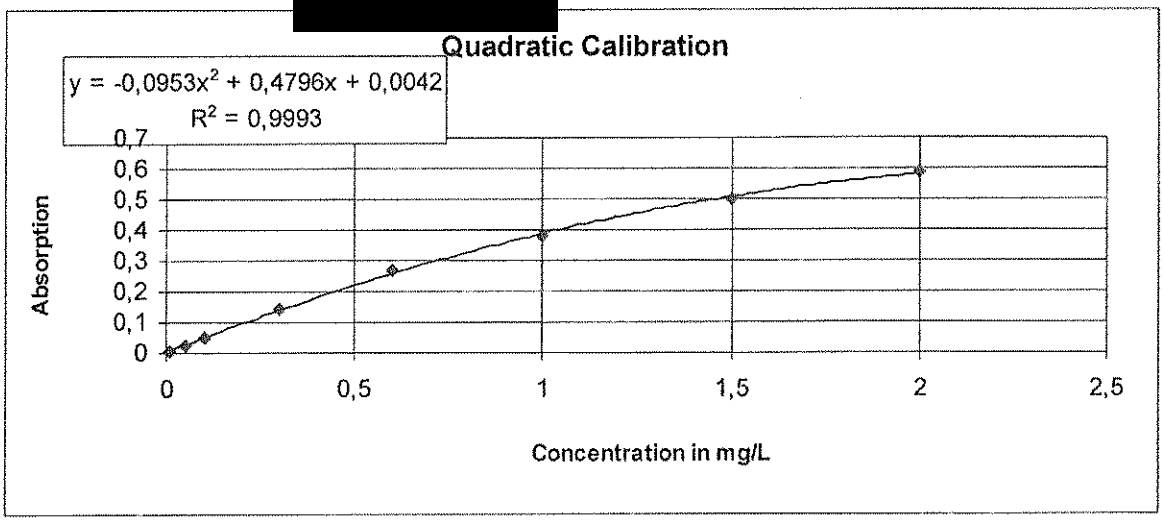
Time	Conc. [redacted]mean 1	Conc. [redacted]ean 2	Mean
0 h	0.4453	0.4476	0.44645
48 h	0.4694	0.4671	0.46825
Stability (= Mean 48h / Mean 0h)			**105 %**

6.7.7 Calibration Curves

6.7.7.1 Calibration of [redacted]



6.7.7.2 Calibration of [redacted]



For each run, the settings and parameters were documented.
All AAS data are archived following GLP regulations.

7 CONDUCT OF THE STUDY

7.1 Selection of Daphnia

24 hours before the start of the test, the adult animals were separated from the young. 23 hours later, the adults were caught with the help of a glass tube, and the newborn daphnia, aged between 0 and 23 hours, were sieved from the medium and immediately placed into a 250 mL-beaker containing dilution water. After a settling-in period of 30 minutes, animals which showed no apparent damage were used for the test.

7.2 Study Performance

Using a glass tube (diameter approx. 8 mm), the daphnia were caught and lifted from the beaker. They were put on a small sieve, and the medium surrounding the animals was sucked off using absorbent paper. Immediately after that, the animals were put into the respective test solution.

The test vessels were left to stand for 48 hours. After 24 and 48 hours, the immobilised daphnia were counted. Daphnia are considered immobilised if they perform no movements or are only able to move their antennae when the beaker is gently agitated. Daphnia which are trapped at the surface of the test solution are also considered immobilised.

The pH, the concentration of dissolved oxygen and the content of the test item in the test vessels were measured at the beginning and at the end of the test.

All values were documented in the raw data.

7.3 Experimental Conditions

The following experimental conditions apply to all studies.

Test vessels	glass beakers, nominal volume 50 mL, tall shape
Feeding	none
Lighting	none

7.4 Main Study

7.4.1 Preparation of the Test Solutions

The saturated solution was prepared by weighing of the nominal load, adding the corresponding amount of dilution water and shaking vigorously for 24 hours. The resulting solution was filtrated through 0.45 µm filters.

7.4.2 Study Parameters

Date of performance	[redacted]
Treatment	10 / 18 / 32 / 56 / 100 mg/L
Temperature	18.1 – 18.4 °C
Duration	48 hours
Observation times	24 and 48 hours
Replicates	four vessels, each containing 20 mL test solution and five daphnia
Control	four vessels, each containing 20 mL dilution water and five daphnia

7.5 Reference Test

7.5.1 Preparation of the Test Solutions

A stock solution containing 250 mg $K_2Cr_2O_7$ /L in deionised water was diluted with dilution water to give a series of concentrations ranging from 0.5 to 3.0 mg/L.

7.5.2 Study Parameters

Date	[redacted]
Treatments	0.5 / 0.625 / 1.0 / 1.25 / 1.5 / 2.0 / 2.5 / 3.0 mg/L
Temperature	22.7 – 24.1°C
Duration	24 hours
Observation time	24 hours
Replicates	four vessels, each containing 20 ± 5 mL test solution and five daphnia
Control	four vessels, each containing 20 ± 5 mL dilution water and five daphnia

8 FINDINGS

8.1 Main Study

8.1.1 Immobilities

In the control, none of the daphnia died or showed any signs of abnormal behaviour throughout the test (see table below).

Table 8.1-a Immobilities

Nominal Concentration in mg/L	Immobility 24 hours					Immobility 48 hours				
	abs.				in %	abs.				in %
0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	1	0	1	10
56	0	0	0	0	0	0	0	2	0	10
100	0	0	1	1	10	3	2	3	1	45

8.1.2 pH and O_2, Temperature

The pH values in the test media and the control ranged from 6.7 to 7.6. The concentration of dissolved oxygen stayed above 8.1 mg/L throughout the test. Temperature range was 18.1 – 18.4 °C. The details are given in the following tables:

Table 8.1-b pH and O_2-values

Nominal Concentration in mg/L	pH		O_2-Concentration in mg/L	
	0 h	48 h	0 h	48 h
0	7.6	7.6	9.0	8.1
10	7.4	7.5	9.3	8.2
18	7.4	7.5	9.3	8.3
32	7.2	7.4	9.2	8.2
56	7.1	7.4	9.5	8.2
100	6.7	7.3	9.2	8.1

Table 8.1-c Temperature

Temperature in °C		
0 h	24 h	48 h
18.4	18.1	18.4

8.1.3 Analytical Determinations

The content of [redacted] part of the test item, [redacted] in the test solution was analytically determined using AAS. Because of the poor solubility in test medium, the correlation between nominal and measured concentration was poor. Therefore, the geometric mean of the measured concentrations was used for the determination of the results. Geometric mean is calculated by multiplication of the n participating concentrations and taking the n^{th} root.

The details are given in the following table:

Table 8.1-d Measured Concentrations in mg/L and corresponding Recovery Rates in %

Nom. Conc. mg/L	Conc. [redacted] mg/L 0h	Conc. [redacted] mg/L 48h	Conc. Test Item mg/L 0h	Conc. Test Item mg/L 48h	% of Nom. Conc. 0h	% of Nom. Conc.48h	% Recovery	Geom. Mean. mg/L
0	< LOQ	< LOQ	--	--	--	--	--	--
10	0.56	0.60	4.2	4.5	42%	45%	107%	4.4
18	1.21	0.94	9.1	7.1	51%	39%	78%	8.1
32	1.79	1.82	13.5	13.7	42%	43%	102%	13.6
56	3.23	3.23	24.4	24.4	44%	44%	100%	24.4
100	5.65	5.56	42.6	42.0	43%	42%	98%	42.3

LOQ = Limit of Quantification (0.01 mg/L)

8.1.4 Biological Results Test Item

The biological results are presented in the following table:

Table 8.1-e Biological Results Test Item

Parameter	Value	95%-confidence interval
NOEC 24h	≥ 42 mg/L	not determinable
24h $EC50_i$	> 42 mg/L	not determinable
24h $EC100_i$	> 42 mg/L	not determinable
NOEC 48h	24 mg/L	not determinable
48h $EC50_i$	> 42 mg/L	not determinable
48h $EC100_i$	> 42 mg/L	not determinable

8.2 Reference Test

The pH values in the test media and the control ranged from 7.4 to 7.6. The concentration of dissolved oxygen stayed above 8.3 mg/L throughout the test. Temperature range was 22.7 – 24.1 °C. The details are given in the following tables:

8.2.1 Tables

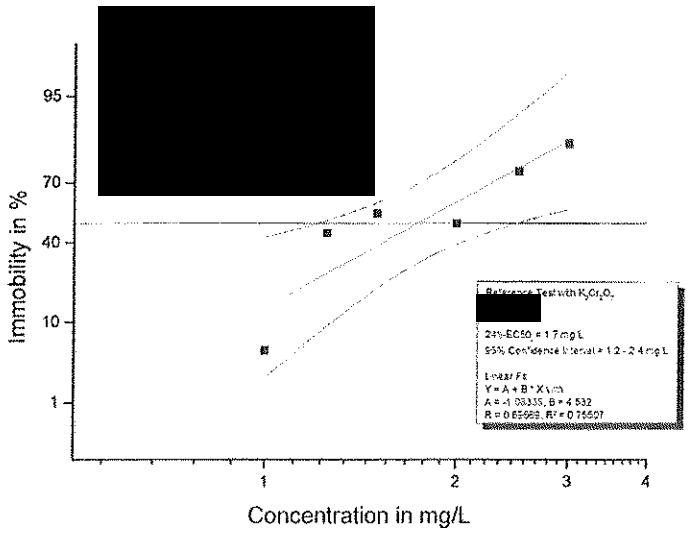
Table 8.2-a Immobilities, pH and O_2-values

Nom. Conc. in mg/L	Immobility					pH		O_2-Concentration in mg/L	
	abs.				in %	t = 0 h	t = 24 h	t = 0 h	t = 24 h
0	0	0	0	0	0	7.6	7.4	8.3	8.3
0.5	0	0	0	0	0	7.6	7.4	8.4	8.4
0.625	0	0	0	0	0	7.6	7.4	8.4	8.4
1.0	1	0	0	0	5	7.6	7.4	8.4	8.4
1.25	1	4	3	1	45	7.5	7.4	8.4	8.4
1.5	4	3	1	3	55	7.5	7.4	8.4	8.4
2.0	3	2	4	1	50	7.5	7.4	8.5	8.4
2.5	3	5	3	4	75	7.5	7.4	8.5	8.4
3.0	4	4	4	5	85	7.5	7.4	8.4	8.4

Table 8.2-b Temperature

Temperature in °C	
0 h	24 h
22.7	24.1

8.2.2 Graph



8.2.3 Biological Results Positive Control

The estimation of the EC50 of the positive control was accomplished using the software Origin™. The calculated values for r resp. r^2 are given in the graph.

The data were evaluated using linear fit on a probability-logarithmic scale.

Equation: Prob.(Y) = A + B * log (X)

The biological results are presented in the following table:

Table 8.2-c Biological Results Positive Control

Parameter	Value	95%-confidence interval
NOEC 24 h	1.0 mg/L	not determinable
24h $EC50_i$	1.7 mg/L	1.2 – 2.4 mg/L
24h $EC100_i$	> 3.0 mg/L	not determinable

9 VALIDITY

- The 24h-$EC50_i$ of $K_2Cr_2O_7$ should lie between 0.6 and 1.7 mg/L.
  The 24h-$EC50_i$ of $K_2Cr_2O_7$ was determined as 1.7 mg/L.
- Immobilisation in the controls may not exceed 10 %.
  Immobilisation in the controls was 0 %.
- The concentration of dissolved oxygen at the end of the test must be at least 3 mg/L.
  The lowest concentration of dissolved oxygen at the end of the test was 8.1 mg/L.

10 DISCUSSION

All validity criteria were met. For the estimation of the EC50s of the positive control, the fits showed sufficient statistical correspondence of the data with the dose-response-equation.

The 24h-$EC50_i$ of potassium dichromate was determined as 1.7 mg/L, lying within the demanded range of 0.6 – 1.7 mg/L.

As the test item is poorly soluble in water, a saturated solution was prepared. This was done by shaking the nominal load with the appropriate amount of dilution water for 24 hours, followed by membrane filtration. In the main study only the highest concentrated treatment showed significant immobilisation (45 %).

At the beginning and at the end of the test, the content of [redacted]omponent of the test item) in the test solution was analytically determined using AAS. Because of the [redacted] in test medium, the correlation between nominal and measured concentration was poor. Therefore, the determination of the results was based on the geometric mean of the measured concentrations.

No observations were made which might cause doubts concerning the validity of the study outcome.

11 DEVIATIONS

11.1 Deviations from the Study Plan

The following deviation from the study plan was documented:

- The temperature for the reference study lay in a range of 22.7 to 24.1 °C. Therefore, the temperature and the deviation within the temperature range were higher than stated in the study plan. As no immobility occurred during the test, this deviation was stated as uncritical.

The deviation was signed and assessed by the study director on [redacted]

- In order to guarantee that the measured concentrations lies in the calibrated range, treatment 56 mg/L was diluted 1:5 and treatment 32 mg/l was diluted 1:2.

The deviation was signed and assessed by the study director on [redacted]

11.2 Deviations from the Guideline

See above.

12 RECORDING

One original of study plan and final report, respectively, all raw data of the study and all documents mentioned or referred to in study plan or final report will be kept in the GLP Document Archive of the test facility for fifteen years. After that, the sponsor's instructions will be applied (shipment of documentation to sponsor). A retain sample of the test item will be kept in the GLP Substance Archive for fifteen years; then, the retain sample will be discarded.

Number of originals which will be sent to the sponsor: 1

13 ANNEX 1: COPY OF GLP-CERTIFICATE

RheinlandPfalz
LANDESAMT FÜR UMWELT, WASSERWIRTSCHAFT UND GEWERBEAUFSICHT

GUTE LABORPRAXIS – GOOD LABORATORY PRACTICE

GLP-BESCHEINIGUNG
STATEMENT OF GLP COMPLIANCE

gemäß/according to § 19b Abs. 1 Chemikaliengesetz

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EC at:

Prüfeinrichtung / Test facility

[redacted]

Prüfung na[redacted]rtise
(gemäß / according ChemVwV-GLP Nr. 5.3/OECD guidance)
1, 3, 4, 5, 6 , 8, 9 (toxikologische in Vitro Prüfungen an Säugerzellen und Bakterien)

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)
29. und 30. November 2010

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that the test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Eine erneute behördliche Überprüfung der Einhaltung der GLP-Grundsätze durch die Prüfeinrichtung ist so rechtzeitig zu beantragen, dass die Folgeinspektion spätestens vier Jahre nach dem Beginn der o.g. Inspektion stattfinden kann. Ohne diesen Antrag wird die Prüfeinrichtung nach Ablauf der Frist aus dem deutschen GLP-Überwachungsprogramm genommen und diese GLP-Bescheinigung verliert ihre Gültigkeit.

Verification of the compliance of the test facility with the Principles of the GLP has to be applied for in time to allow for a follow-up inspection to take place within four years after commencing the above mentioned inspection. Elapsing this term, the test facility will be taken out of the German GLP-Monitoring Programme and this GLP Certificate becomes invalid.

Unterschrift, Datum / Signature, Date

Dr.-Ing. Stefan Hill - Präsident -
(Name und Funktion der verantwortlichen Person / name and function of responsible person)

Landesamt für Umwelt, Wasserwirtschaft und Gewerbeaufsicht
Kaiser-Friedrich-Straße 7, 55116 Mainz
(Name und Adresse der GLP-Überwachungsbehörde / Name and adress of the GLP Monitoring Authority)

HESSEN ATWERTEN BERATEN

14 ANNEX 2: COMPOSITION OF M4-MEDIUM

Parameter	Concentration	
$CaCl_2*2H_2O$	293.80	mg/L
$MgSO_4*7H_2O$	123.30	mg/L
$NaHCO_3$	64.80	mg/L
KCl	5.80	mg/L
$NaNO_3$	274	µg/L
K_2HPO_4	184	µg/L
KH_2PO_4	143	µg/L
$Na_2SiO_3*9H_2O$	10	mg/L
H_3BO_3	2.8595	mg/L
$Na_2EDTA*2H_2O$	2.5	mg/L
$FeSO_4*7H_2O$	0.9955	mg/L
$MnCl_2*4H_2O$	0.3605	mg/L
LiCl	0.306	mg/L
$SrCl_2*6H_2O$	0.152	mg/L
RbCl	0.071	mg/L
$Na_2MoO_4*2H_2O$	0.0615	mg/L
$CuCl_2*2H_2O$	16.75	µg/L
NaBr	16	µg/L
$ZnCl_2$	13	µg/L
$CoCl_2*6H_2O$	10	µg/L
KI	3.25	µg/L
Na_2SeO_3	2.19	µg/L
NH_4VO_3	0.575	µg/L
ThiaminHCl	75	µg/L
Cyanocobalamin	1	µg/L
D+Biotin	0.75	µg/L

Deionised water is used as base of M4.